

Maize Phenology Affects Establishment, Damage, and Development of the Western Corn Rootworm (Coleoptera: Chrysomelidae)

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ABSTRACT The effects of maize (*Zea mays* L.) phenology on establishment and adult emergence of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and plant damage to maize was evaluated in field trials in 2001 and 2002 and in the greenhouse. Although neonate western corn rootworm larvae were able to initially establish on maize roots during anthesis and early reproductive stages, these older roots were apparently unsuitable for complete western corn rootworm larval development. The number of western corn rootworm beetles that emerged from eggs that hatched during anthesis to early reproductive stages was significantly fewer than the number of beetles that eclosed from V4 to V11. Plant damage was also lowest from larvae that eclosed during anthesis to early reproductive stages. Potential causal mechanisms and implications of these data in terms of potential management strategies in the future are discussed.

KEY WORDS *Diabrotica virgifera virgifera*, phenology, root toughness

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a primary economic pest of maize in the U.S. Corn Belt and costs growers more than \$1 billion annually in control costs and yield losses (Metcalf 1986, Rice 2004). The larval stage of the insect is by far the most damaging, because larvae feed on maize roots, reducing the ability of the plant to uptake water and nutrients (Kahler et al. 1985). Grain yield reductions result from the disruption of root system function because of larval feeding (Branson et al. 1980, Spike and Tollefson 1988, Riedell et al. 1991, 1992, 1996, Spike and Tollefson 1991, Riedell and Evenson 1993, Godfrey et al. 1993). Larval feeding wounds may also increase the likelihood of root and stalk rot fungus infection (Palmer and Kommedahl 1969). In even moderate infestations, plants are more susceptible to lodging in wind and rain because of root damage. Adults may also damage maize plants by feeding on the silks, ear tips, kernels, and foliage, although economic damage from adult feeding is rare except at high population densities in maize seed production (Chiang 1976, Capinera et al. 1986).

Strnad and Bergman (1987) determined distribution of western corn rootworm larvae in maize roots. First-instar larvae were found in the cortex of seminal and all nodal roots, with the majority of larvae in roots with a diameter of 2.0 mm or less. Of particular interest for this study, they observed that, over the course of a growing season, larvae tended to move toward newly

developed nodal roots, which are larger diameter roots previously not available for feeding. Their work suggests that older western corn rootworm larvae prefer newly developed, larger diameter nodal roots to more mature roots, but it is not known if young roots are needed for continued development.

Stavisky and Davis (1997) conducted field experiments during the 1994 and 1995 field seasons in New York State to quantify the effects of maize maturity class on larval establishment and adult emergence. Early, middle, and late maturing maize varieties (Pioneer Brand 3979, 3769, and 3394, respectively) were evaluated for larval survival and adult emergence. The authors found that the average number of larvae recovered per plant did not differ significantly with variety. An interesting sideline of their results showed that recovery of first-instar western corn rootworm larvae occurred over a shorter period of time on the early maturing variety compared with the middle and late maturing maize varieties. Approximately 66% more adults were recovered from the late maturing variety compared with the early maturing variety. Late in the season, adults were not recovered from the early maturing variety. One possible explanation is that late hatching larvae on the early maturing variety may have been less able to complete development as evidenced by few adults emerging late in the season from these plants. The longer root development time for the late maturing variety may have resulted in a longer period where larval development was possible, extending the seasonal occurrence of the western corn rootworm.

An understanding of western corn rootworm larval establishment on host plants is of particular importance for the development of western corn rootworm

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control recommendations and potentially integrated resistance management programs. Because planting date influences western corn rootworm seasonal population dynamics (Musick et al. 1980, Bergman and Turpin 1984, 1986), infestation at different plant stages (i.e., maize phenologies) should yield valuable information on the interaction between the insect and host plant.

The goal of this project was to further understanding of the relationships between larval establishment, plant damage, and adult emergence at differing maize phenologies.

Materials and Methods

Field Experiments

Field experiments were conducted in 2001 and 2002 at the University of Missouri Bradford Research and Extension Center, located 9.6 km east of Columbia, MO. One maize variety, Pioneer Brand 3394, was used for all experiments. Cultural practices (tillage, fertilization, herbicide application, etc.) were typical of agricultural procedures for the area. The soil type was Mexico silt loam with 2% sand, 70% silt, and 28% clay, as determined by the MU Soils Testing Laboratory. Each year, the field chosen was planted to soybean, *Glycine max* L., the previous year. Oviposition outside of maize by western corn rootworm adults had not been detected in Missouri at the time of these experiments, so we assumed that the western corn rootworm larvae and adults collected from these field plots were artificially infested. To verify this assumption, an uninfested control was included.

The field experiments were planted on 20 April 2001 and 18 April 2002 in plots that were 6.4 m long and eight rows wide with 0.91-m row spacing and 21.6-cm plant spacing using a mechanical planter (Wintersteiger/Hege, Salt Lake City, UT). The experiment was set up in a 10 by 3 (infestation date \times infestation level) factorial treatment arrangement in a randomized complete block, split-plot design in space as outlined in Steel et al. (1997). The main plot was infestation date and the subplot was infestation level. Infestation date was randomly assigned to 1 of 10 ranges within each of five replications.

Within the above experimental design, there were four sample types, one per infested row. One uninfested row between infested rows provided a buffer to prevent across-row larval movement (Hibbard et al. 2003, 2004). The order of the rows used for each sample type was randomized within each replicate and infestation date. The order of infestation level was also randomized within each infested row. Individual plants for each infestation level were infested in the portion of the field set aside for that infestation date and that replication. Within each plot, each infested row contained plants assigned to all three infestation levels.

Infestation started at planting and continued for nine additional infestation dates at approximately weekly intervals. In each infested row, three plants at

least 1 m apart were infested with 0, 200, or 600 viable western corn rootworm eggs from the primary diapausing colony at the USDA-ARS Laboratory in Brookings, SD. For each infestation date each year, eggs were removed from cold storage just before washing and infestation in the field. The percentage of viable eggs from a series of subsamples of all eggs was determined 1 mo before planting each year by allowing eggs to hatch on sterilized, moist filter paper. The actual number of eggs was adjusted so that 200 or 600 viable eggs were infested per plant. Plants were infested by injecting 10 ml of a 0.15% agar solution into each of two 1.3-cm-diameter by 5.1-cm-deep holes, one on each row side of the plant, \approx 5.1–7.6 cm from the base of the plant using a 50-ml glass syringe. The zero viable eggs per plant infestation consisted of the agar solution without eggs. After infestation, the holes were covered with soil. Maize phenology (Ritchie et al. 1992) was recorded at each infestation date and when samples were taken from the field.

Four sample types were collected and analyzed separately: larval recovery at \approx 370 DD after infestation (L1, most larvae at early second-instar stage), larval recovery at \approx 440 DD (L2, most larvae at early third-instar stage), root damage at \approx 570–620 DD (about one half the larvae had pupated), and adult emergence. For larval sampling (L1 or L2), we used the technique described by Hibbard et al. (2004). Briefly, root balls with associated soil were removed from the field in plastic mesh "onion" bags (Sacramento Bag Manufacturing, Woodland, CA). The onion bags were hung over water pans in a greenhouse bay with the cooling system mostly off for a minimum of 7 d where temperatures routinely exceeded 50°C during the day. This technique, under Missouri summer conditions, is analogous to use of a Tullgren funnel. Western corn rootworm larvae fell from the drying samples into the water pans set below. Most larvae were recovered within the first 4 d using this technique, with larval recovery usually peaking on days 2 and 3. Occasionally, especially when roots were sampled just after a rain event and/or when cool, cloudy days followed root collection, larvae were recovered up to day 6. Larvae were collected one or more times daily and stored in 95% ethanol until processing. In the laboratory, each larva was examined under a microscope for urogomphi to differentiate southern corn rootworm larvae, *Diabrotica undecimpunctata howardi* Barber, (urogomphi present) from western corn rootworm larvae (urogomphi absent) (Krysan 1986). Any southern corn rootworm larvae collected from samples were tallied and discarded, and the total number of western corn rootworm larvae recovered was recorded.

Root damage samples were taken at the point of maximum damage, when it was estimated that one half of the larvae had pupated. This averaged \approx 570–620 DD after infestation. Roots were removed from the field, washed the same day, and damage rated using the 0–3 node injury scale (Oleson et al. 2005). Adult emergence cages, similar in design to Hein et al. (1985), were placed over infested plants randomly

assigned for adult emergence. Modifications (Pierce and Gray 2007) allowed the maize plant to remain alive and grow throughout the adult emergence monitoring period. Traps were placed over the plants during the whorl stage and set up to collect insects (stalk sealed and collecting jars on at ≈ 550 –600 DD). Adult insects were collected three times per week and were stored in 95% ethanol until processing. Total number, sex, head capsule width (2002 only), and dry weight were recorded for all western corn rootworm adults collected. Southern corn rootworm adults were tallied and discarded.

Degree-day accumulation began on the day of infestation and was calculated by subtracting 11.1°C , the developmental threshold (Wilde 1971, Levine et al. 1992), from the average 24-h bare soil temperature at a depth of 5.08 cm. Soil temperature data were obtained from the University of Missouri Commercial Agriculture Automated Weather Station Network station located at the Missouri Agricultural Experiment Station (South Farm), 6 km southeast of Columbia, MO. Because maize phenology at the time of egg hatch is more relevant than maize phenology at the time of infestation, egg hatch was estimated as 300 DD under Central Missouri conditions, and maize phenology at this time is reported.

Greenhouse Experiments

To help substantiate observed field effects under more controlled conditions, a greenhouse experiment was conducted at the USDA-ARS greenhouse facility located on the University of Missouri-Columbia campus. The greenhouse experiment was initiated on 21 August 2002 and was set up in a randomized complete block design with five replicates. Four samples were taken from each of 10 infestation dates (two larval recovery times, plant damage, and adult emergence for a total of 200 pots). All pots were 19-liter black plastic pots with fine ($114\text{-}\mu\text{m}$ opening) stainless steel mesh screens (TWP, Berkeley, CA) affixed over the drain holes from the inside of each pot using hot glue to prevent larvae from exiting the experiment (Clark and Hibbard 2004). The two larval recovery samples (L1 and L2) were taken ≈ 1 and 2 wk after infestation with neonate larvae, respectively. Damage samples were collected ≈ 3 wk after infestation.

Before planting, the pots were filled with a planting medium that was 2:1 (vol:vol) autoclaved soil to peat-based growing medium (Pro-Mix; Premier Horticulture, Red Hill, PA). Each pot was planted with two kernels of maize variety Pioneer Brand 3394. Because live larvae were infested, plants were allowed to germinate for 1 wk before the first infestation. Just before the first infestation date, all pots were thinned to one plant per pot. Plants were watered when the soil surface was dry and fertilized weekly starting 2 wk after planting with Peters Professional general purpose 20–20–20 fertilizer (Scotts-Sierra Horticultural Products, Marysville, OH) at the recommended rate of 1.0 g/3.8 liters water. The greenhouse was maintained at average temperature of $\approx 25.0 \pm 2.0^{\circ}\text{C}$. Supplementary lighting was provided to adjust the photoperiod to

$\approx 14:10\text{-h}$ light:dark with 1,000-W sodium bulbs (GE Lighting, Cleveland, OH).

Twenty plants were hand infested weekly for 10 consecutive wk with 30 neonate western corn rootworm larvae that were 24 h old or less. This infestation level is roughly equivalent to infesting ≈ 600 viable eggs in the field, because $\approx 5\%$ of viable eggs become established on a host plant (Hibbard et al. 2004). However, because only $\approx 50\%$ of neonate larvae establish on host plants in greenhouse situations (Clark and Hibbard 2004), the infestation level was actually more similar to infesting 300 viable eggs in the field. Newly hatched larvae were obtained from eggs produced by a nondiapausing laboratory colony maintained in the greenhouse facility. These were sometimes supplemented from the nondiapausing colonies at USDA-ARS NGIRL, Brookings, SD, the original source of our colony. Neonate larvae were manually transferred using a damp fine-tipped nylon brush into a shallow trough ≈ 2.5 cm from the base of the plant. After infestation, the trough was carefully covered with loose soil.

For larval recovery, the soil from each pot was divided into thirds and placed in modified Berlese funnels placed under 60-W light bulbs. Under our conditions, preliminary experiments showed 95% of those that were recovered (90 total larvae) were recovered within 72 h. Five additional larvae were recovered over the following 168 h. Finally, five larvae were not recovered. For these experiments, samples were placed in the apparatus for at least 72 h. Larvae recovered were preserved in ethanol for further processing in the laboratory. Larvae collected from a single plant were counted and combined for wet and dry weight determination.

For adult emergence, a hole was cut in the center of a nylon mesh, and the screens were carefully placed over the plant, secured to the stalk with a cable tie, and held to the pot with a large rubber band just before predicted adult emergence initiation (3.5–4 wk after infestation). The soil was kept moist and the pots were checked daily for adults by lifting the screens from the pots. Adults recovered from each pot were tallied daily and preserved in 95% ethanol until processing.

Statistical Analysis

PROC MIXED of the SAS statistical package (SAS Institute 1990) was used for data analysis. A separate analysis was done for larval recovery (L1 and L2 were combined), plant damage, and adult emergence for the field trials. Field data from 2001 and 2002 were combined for all three analyses. Before conducting the combined analysis, a test of homogeneity of error mean squares for both years was done as outlined by Snedecor and Cochran (1989). Although in both years there were 10 infestation dates, the maize phenology of the 10 dates did not match up when analyzing years together. However, when infestation dates 1 and 2, 3 and 4, 5 and 6, 7 and 8, and 9 and 10 were pooled, maize phenology between the 2 yr did match up, and these five groupings of infestation dates were used in the

Table 1. Number of western corn rootworm larvae recovered, plant damage, and no. of adults emerged (mean \pm SE) when infested at different maize phenologies averaged across 2001 and 2002^a

Maize phenology ^b	200 eggs/plant			600 eggs/plant		
	Larvae ^c	Damage ^d	Adults	Larvae	Damage	Adults
V4-6	9.2 \pm 2.0 c	0.16 \pm 0.04 bc	3.4 \pm 1.1 bc	19.6 \pm 2.3 bc	0.38 \pm 0.09 b	7.4 \pm 2.2 bc
V6-7	18.9 \pm 4.5 a	0.20 \pm 0.05 b	9.6 \pm 1.8 ab	27.6 \pm 6.3 b	0.84 \pm 0.14 a	13.7 \pm 2.8 ab
V8-9	9.4 \pm 1.9 bc	0.49 \pm 0.13 a	14.6 \pm 3.4 a	11.4 \pm 2.2 c	0.65 \pm 0.16 a	17.7 \pm 5.2 a
V9-11	7.7 \pm 1.6 c	0.23 \pm 0.05 b	5.5 \pm 1.1 bc	15.6 \pm 2.7 c	0.38 \pm 0.12 b	6.8 \pm 2.0 c
VT-R1	17.6 \pm 3.4 ab	-0.02 \pm 0.06 c	-0.05 \pm 0.14 d	39.2 \pm 8.6 a	0.16 \pm 0.08 c	0.2 \pm 0.3 d

^a Data from the 0 infestation level was subtracted from data from the 200 and 600 infestation levels for each replication of each trait. In addition to averaging all data across 2001 and 2002, the no. of larvae recovered was also averaged across two sample times (370 and 440 DD after egg infestation, 11°C threshold) for each replication.

^b Approximate maize phenology at the time of egg hatch as predicted by growing degree days from infestation day.

^c Significant differences within a column ($\alpha \leq 0.05$) calculated from the *t*-test output of the SAS mixed model are indicated by different lowercase letters.

^d Root damage ratings following Oleson et al. (2005).

analysis. Larval recovery, plant damage, and adult emergence from the zero eggs per plant infestation level were random and low. Instead of including these data in the analyses and tables, before the analysis, any damage, adult emergence, or larvae recovered from the zero eggs per plant infestation level was subtracted from the data from both the 200 and 600 eggs per plant infestation level within the particular infestation date and replication. Treatments for adult emergence and plant damage were arranged as a 2 by 5 by 2 (years \times infestation dates \times egg infestation levels) factorial, and data were analyzed as a randomized complete block split-plot in space as outlined in Steel et al. (1997). The linear statistical model contained the main plot effect of years, the subplot effect of infestation dates, egg infestation levels, and all possible interactions. Replications within year served as the denominator of *F* for testing the effects of year. Replications within year, infestation dates, and egg infestation levels served as the denominator of *F* for testing infestation dates and the interaction of infestation dates and egg infestation levels. All other effects used the residual error for the denominator of *F*. Beyond the standard analysis of variance (ANOVA), we pre-planned comparisons of infestation dates within egg infestation levels, and this was done with the *t*-test output from PROC MIXED. Field data for larval recovery were analyzed as above, but one additional factor, larval recovery time, and its interactions was added to the model. Greenhouse data were analyzed as a simple randomized complete block experiment for adult emergence and damage and as a randomized complete block split-plot for larval recovery (with larval sample time as the subplot). Comparisons of infestation dates was done with the *t*-test output from PROC MIXED.

Results

Field Study

Adult Emergence. Maize phenology at the time of western corn rootworm egg hatch significantly impacted the ability of western corn rootworm larvae to survive to the adult stage ($F = 14.41$; $df = 4,72$; $P <$

0.0001). The number of adults that emerged from eggs that hatched during VT to R1 (Ritchie et al. 1992) was significantly lower than the number of adults that emerged from eggs which hatched during all earlier stages of maize development for both the 200 and 600 eggs infestation levels (Table 1). The greatest number of adults emerged from eggs that hatched during V8 to V9, followed by eggs that hatched during V6 to V7 (Table 1). Egg hatch during V4 to V6 and egg hatch during V9 to V11 produced significantly more adults than eggs that hatched from VT to R1, but significantly fewer adults than eggs that hatched during V8 to V9. The effect of egg infestation level, year of the study, and the interaction of egg infestation level and maize phenology did not significantly impact the number of adults emerged, nor did the interaction of year with any of the above factors. Beetle sex, head capsule width, and dry weight did not seem correlated with maize phenology at egg hatch and data are not shown.

Plant Damage. Damage to maize roots was significantly impacted by plant phenology at the time of western corn rootworm egg hatch ($F = 14.27$; $df = 4,72$; $P < 0.0001$). Year of the study, the interaction of year by egg infestation level, and the three-way interaction of year by egg level by maize phenology did not significantly affect plant damage, but egg infestation level ($F = 31.09$; $df = 1,72$; $P < 0.0001$), the interaction of egg infestation level and maize phenology ($F = 3.89$; $df = 4,72$; $P = 0.0065$), and the interaction of year and maize phenology ($F = 16.83$; $df = 4,72$; $P < 0.0001$) did significantly impact plant damage. Plant damage was lower when eggs hatched during VT to R1 than for egg hatch time during any earlier maize phenology and significantly so for the 600 eggs per plant infestation level (Table 1). Plant damage was highest when egg hatch was during the V6 to V7 stage when infested with 600 eggs per plant and at V8 to V9 when infested with 200 eggs per plant (Table 1).

Larval Recovery. The number of larvae recovered from maize root balls was affected by maize phenology ($F = 14.16$; $df = 4,72$; $P < 0.0001$), egg infestation level ($F = 29.50$; $df = 1,72$; $P < 0.0001$), number of degree-days after infestation ($F = 22.05$; $df = 1,80$; $P < 0.0001$), years of the study ($F = 13.43$; $df = 1,8$; $P < 0.0064$), the

Table 2. Number of western corn rootworm larvae recovered, plant damage, and no. of adults emerged (mean \pm SE) when infested at different maize phenologies in the greenhouse

Neonate infestation date	Number of larvae recovered ^a	Plant damage	Number of adults recovered
V2	2.2 \pm 0.7 c	0.42 \pm 0.14 a	2.0 \pm 0.6 c
V3	7.4 \pm 1.8 ab	0.51 \pm 0.16 a	6.8 \pm 2.2 abc
V5	9.8 \pm 2.0 a	0.46 \pm 0.18 a	7.6 \pm 2.7 abc
V7	6.8 \pm 1.4 ab	0.51 \pm 0.16 a	11.0 \pm 2.0 a
V8	8.1 \pm 1.5 ab	0.38 \pm 0.11 ab	10.6 \pm 2.2 ab
V10	4.0 \pm 0.6 c	0.08 \pm 0.02 bc	5.0 \pm 2.0 bc
V13	5.9 \pm 1.6 b	0.08 \pm 0.01 c	4.0 \pm 2.0 c
V18	4.2 \pm 1.3 c	0.05 \pm 0.01 c	5.0 \pm 1.8 bc
R1	6.5 \pm 0.9 b	0.05 \pm 0.01 c	4.4 \pm 2.9 c
R2	7.6 \pm 1.4 ab	0.03 \pm 0.01 c	2.0 \pm 0.7 c

^a Significant differences within a column calculated from the *t*-test output of the SAS mixed model are indicated by different lowercase letters are indicated by different lowercase letters.

interaction of year by maize phenology ($F = 20.72$; $df = 4,72$; $P < 0.0001$), the interaction of year by egg infestation level ($F = 8.86$; $df = 1,72$; $P < 0.0040$), the interaction of maize phenology and egg infestation level ($F = 2.92$; $df = 4,72$; $P < 0.0270$), and the three-way interaction of maize phenology, egg infestation level, and year ($F = 5.76$; $df = 4,72$; $P = 0.0004$). Although egg hatch during VT to R1 resulted in significantly fewer adults and less damage than egg hatch at earlier maize phenologies, the number of larvae recovered was actually significantly greater from this egg hatch time than from any earlier egg hatch time for the 600 eggs per plant infestation level (Table 1). For the 200 eggs per plant infestation level, the number of larvae recovered when larvae hatched during the VT to R1 maize phenology was not significantly fewer than the maize phenology with the greatest number of larvae recovered, the V6 to V7 stage (Table 1). For both the 200 and the 600 eggs per plant infestation levels, there appeared to be two peaks, in terms of the number of larvae recovered: V6 to V7 and VT to R1. Larval head capsule width and dry weight did not seem correlated with maize phenology at the time of egg hatch and data are not shown.

Greenhouse Study

Maize phenology significantly affected the number of adults emerged ($F = 2.42$; $df = 9,36$; $P = 0.0287$), plant damage ($F = 2.42$; $df = 9,36$; $P = 0.0287$, and the number of larvae recovered ($F = 3.90$; $df = 9,36$; $P = 0.0016$). Adult emergence peaked when infested with neonate larvae at V7. Significantly more adults were produced than when infested with neonate larvae at V2, V10, V13, V18, R1, or R2 (Table 2). Plant damage did not differ significantly between any of the five infestation dates between V2 and V8 (Table 2). At V8, plant damage dropped dramatically and again leveled off with only minor damage and no significant difference between infestations at V10, V13, V18, R1, and R2 (Table 2). The average number of larvae recovered peaked when maize was infested with neonate larvae at V5, when significantly more larvae were recovered

than when infested at V2, V10, V13, V18, and R1 (Table 2). The number of larvae recovered when infested at V5 was not significantly different than the number of larvae recovered when infested at V3, V7, V8, and R2. Size of larvae and adults and beetle sex did not seem to be correlated with maize phenology at infestation, and data are not shown.

Discussion

In this study, although significant numbers of western corn rootworm larvae were recovered when egg hatch in the field occurred during a maize phenology of VT to R1, almost no adults emerged and very little damage resulted (Table 1). Neonate western corn rootworm larvae were able to establish on the older, larger root systems present at VT to R1 but apparently were not able to survive long enough to produce significant damage or adults. It has been known for some time that western corn rootworm larvae move to the newly emerging nodes of roots as they emerge from the stalk (Apple and Patel 1963, Strnad and Bergman 1987). Data from Stavisky and Davis (1997) hinted that late hatching larvae may be less able to complete development on an early maturing maize variety. Data from this study supports the hypothesis that western corn rootworm larvae not only prefer younger roots as they emerge from the stalk, but they also require younger root systems to complete their development.

Timing of planting and/or timing of artificial western corn rootworm egg infestation has been previously evaluated, but not with as many dates as this study. Musick et al. (1980) conducted field studies in much of the Corn Belt to examine the relationship between planting date, adult emergence, and root damage. In general, the authors reported a decrease in total western corn rootworm adult emergence and delayed peak emergence from later planted plots compared with plots planted in late April and early May. Analysis of root rating data indicated that each 10-d delay in planting reduced root damage on the 1–6 scale (Hills and Peters 1971) by 0.6 (Missouri) to 1.1 (Nebraska) units, depending on area. Both Branson and Sutter (1986) and Hibbard et al. (1999) evaluated egg infestation dates at planting, V1–2, and \approx V4. Although soil temperatures were not reported in either study, application times in both of these studies were similar to the first three application times in this study, so egg hatch may be assumed to be roughly similar as well. Branson and Sutter (1986) found plant damage was greatest with the last infestation. Hibbard et al. (1999) found greatest damage with the earliest application of eggs. Differences between the two studies, in part, were likely caused by differences between soil temperatures in South Dakota and Missouri. In this study, we report greatest damage between the third and sixth infestation dates, which were between V1 and V6 at infestation and approximately V6 to V9 at egg hatch (Table 1). Adult emergence also peaked during this time frame.

In addition to maize, other alternate hosts also become less suitable for western corn rootworm development with aging phenology. Chege et al. (2005) evaluated large crabgrass, *Digitaria sanguinalis* L. Scop, giant foxtail, *Setaria faberi* R.A.W. Herrm, witchgrass, *Panicum capillare* L., woolly cupgrass, *Eriochloa gracilis* (J. Presl) Kunth, and green foxtail, *Setaria viridis* L. P. Beauv. in addition to maize. Percentage larval recovery, change in head capsule width, and weight gain were significantly impacted by infestation time and host species. Significantly fewer larvae were recovered from later infestation times than earlier infestation times, and the head capsule width of those that were recovered was significantly smaller than the head capsule width of those larvae that were infested at earlier plant phenologies (Chege et al. 2005).

What factors are present in older roots of host species and not present in younger host roots that could be responsible for the reduced success of western corn rootworm larvae on older roots? We do not know for certain what factors are involved, but Zeier et al. (1999) documented that lignin and suberin (another biopolymer) were found in significantly greater amounts in older portions of maize roots than in younger portions at root tips. Lignin is involved in mechanical support and, because it is difficult to digest, it is also involved in plant defense (Campbell and Sederoff 1996). Lignin level has been correlated to resistance to feeding by the European corn borer, *Ostrinia nubilalis* (Hübner) (Martin et al. 2004). It is possible that older, tougher roots are also not as easy to digest for western corn rootworm larvae. It is also possible that older roots lack nutrition factors that might be found in younger roots. Although it is unlikely that we could take advantage of the knowledge that western corn rootworm larvae do poorly on older roots in the management of this major pest without a more thorough knowledge of the exact factors involved, if the factor(s) were known it may be possible to incorporate factors negative to the western corn rootworm into maize or remove nutritious factors from maize earlier in the season than currently occurs. The recent completion of the maize genome and the advancement of maize genomics may make this possible.

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